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Original Article

Phytochemical, antioxidant, antipyretic and anti-inflammatory activities of aqueous-methanolic leaf extract of *Mangifera indica*

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Abstract: Objective: Plant-based natural antioxidants have a wide variety of biological activities with significant therapeutic value. *Mangifera indica* has been used traditionally to treat a variety of ailments in animals and human, but little is defined about its biological or pharmacological effects. Therefore, the objective of the present study was to evaluate phytochemical, antioxidant, antipyretic and anti-inflammatory activities of aqueous-methanolic leaf extract of *M. indica.* Methods: To investigate the possible impact of aqueous-methanolic leaf extract of *M. indica* on oxidative stress, inflammation, and pyrexia, we used a combined in vitro and in vivo series of experiments on laboratory animals. Results: Results revealed significant antioxidant potential in 2,2-diphenylpicrylhydrazyl (DPPH) and nitric oxide (NO) scavenging assay, while significant but dose dependent antipyretic potential was documented in typhoid-paratyphoid A and B (TAB) vaccine and prostaglandin E (PGE) induced pyrexia models. Significant anti-inflammatory effects were observed in both acute and chronic inflammatory models of arachidonic acid and formalin. Phytochemical screening and high-performance liquid chromatography (HPLC) analysis of *M. Indica* confirmed the presence of mangiferin, quercetin, and isoquercetin. These phytoconstituents likely play a role in the observed biological activities. Our results show that *M. indica* has antioxidant, anti-inflammatory, and antipyretic effects, lending credence to its traditional use and advocating for its utilization as a viable contender in treating oxidative stress-associated ailments. Conclusion: It is concluded that *Magnifera indica* has various properties in the treatment of various diseases.

Keywords: Inflammation, pyrexia, antioxidant, *Mangifera indica*, prostaglandins

Introduction

Medicinal plants are gift from nature to humans. Since ancient times, herbs and spices have been used for medicinal purposes [1, 2]. These plants are primarily used as a crucial component of the therapeutic pillars [2, 3]. Unani, Hikmat, and Tibbe-Nabvi are well-known names for this school of therapy in Pakistan. More than 90,000 registered and unregistered hakims or tabibs use the 1000-1200 medicinal plants for therapeutic and curative purposes [2, 4]. Since this practice is based on personal experiences instead of scientific evidence, it needs to be proven scientifically. Pakistan's National Council for Tibb is working hard to ensure that medic-

inal plants in Pakistan are based on science and do not harm people [4, 5].

Pain and inflammation are an increasing global public health concern [6, 7]. Nonsteroidal anti-inflammatory agents (NSAIDs), corticosteroids, and opioids are commonly used to treat symptoms of pain and inflammation [6, 8]. Still, it is crucial to ensure that drugs can be put on the skin's surface and reach deeper skin tissues by penetration (for example, through massage or ultrasound) [9]. The symptoms of an infection, cancer or another disease can cause fever or pyrexia [10]. The body makes an environment that makes it hard for infectious pathogens or damaged tissues to live [2, 10]. Pro-inflammatory mediators (cytokines such as interleukin 1, 6, and tumor necrosis factor-α) are typically produced rapidly by infected or injured tissue, which increases the production of prostaglandin E_2 (PgE_2) near the brain and triggers the hypothalamus to raise body temperature [2, 11]. Antipyretic medications typically block PgE_2 production to lower the elevated body temperature [11]. These manufactured compounds inhibit cyclooxygenase-2 (COX-2) in a way that cannot be undone, but they are dangerous for the brain, kidneys, heart muscles, and liver cells [2, 7, 12].

Mangifera indica, commonly known as mango (Chaunsa), belongs to the "Anacardiaceae" family, the plant kingdom native to the Indian subcontinent. Hundreds of developed assortments have been presented to other warm-weather countries worldwide [13]. Many countries have used mango extract from leaves, fruit pulp, roots, bark, stem, and seed kernel for curative purposes [14]. The different chemical constituents are present in the leaf of *Mangifera indica*, such as flavonoids, alkaloids, phenols, saponins, minerals, and vitamin C and B [15]. The leaf extract is being used for different biological activities, such as anti-diabetic [16], anti-microbial [17], immunomodulators [18], anti-allergic [19], hepatoprotective [20], cardioprotective [21], anti-inflammatory, and analgesic [22].

Mangiferin is a xanthone in various parts of the *M. indica*, such as the peel, leaves, stalks, kernel, and barks [23]. The aglycone 1,3,6,7-tetrahydroxy-xanthone hydrolysis with R-acetobromoglucose through O-glycosidic bond formation results in the synthesis of mangiferin [24]. It has hepatoprotective, anti-diabetic, anti-viral, anti-aging, anti-cancer, and immunomodulatory effects [23]. Mangiferin reduced lipid peroxidation induced by hydrogen peroxide in human white blood cells [25]. Mangiferin is used to cure various eye diseases [26].

Quercetin (3,3',4',5,7-pentahydroxyflavone) is an essential bioflavonoid in more than twenty plant materials. It cannot be produced naturally in the human body [27]. It has an anti-inflammatory effect. Quercetin is used to treat metabolic ailments, hypertension, prevent cardiac hypertrophy, and chronic heart disease (CHD), and it can control obesity because it can block glucose uptake from the blood, inhibit fat production and accumulation in cells, trigger the apoptosis of existing fat cells, and decrease cholesterol [28].

Isoquercetin (quercetin-3-O-β-D-glucopyranoside) is a natural flavonoid found in various parts of the plant, including herbs, fruits, and vegetables [29, 30]. The conjugation of different glucose moieties makes them more soluble in water, and isoquercetin is easier to get [31].

Materials and methods

Drugs, chemicals, and instruments

Diclofenac sodium was purchased from SAMI Pharmaceuticals (Pvt) Ltd. KRC, SD, Pakistan and Carbpol-940 from Duksan Pure Chemicals Co., Ltd., South Korea. Methanol was purchased from Merck, Germany. Sodium benzoate, triethanolamine, and glycerol were purchased from Sigma-Aldrich, Pakistan. Distilled water was purchased from SARCO Chemicals, Mtn, Pakistan. The thermometer was purchased from Unex Diagnostics, LHR, PU, Pakistan. Analytical grade (all other) chemicals and reagents were used in this experiment for phytochemistry and antioxidant assays.

Collection of plant materials and extract preparation

M. indica was taken from a Multan, South Punjab, at the Muhammad Institute of Medical and Allied Sciences. An expert taxonomist identified the plant at the Department of

Agronomy, MNS University of Agriculture, Multan (R.R.Steward, F.W. Pak. 625-3). The fresh leaves of the plant were left for shade drying. Dirt and debris were cleared before the grinding of dried leaves by the special herb grinder to the coarsely powdered form. The airtight jar was used for the preservation of the powdered plant. For extract, preparation from powdered material was done by a standard reported method, including a maceration procedure in an aqueous-methanolic (70:30) mixture. The evaporation of crude extract pool to a thick paste as stock solutions was done on a rotatory evaporator at 37°C under low pressure [32-35]. The estimated 10% yield of extract was taken using the formula.

% yield = (weight after evaporation × 100)/dry weight of leaves (1)

Its 20%, 10%, and 5% dilutions were stored in airtight jars in a lab refrigerator at -2°C.

Animals

Male local bread rabbits and albino rats were used for experimentation in the laboratory of pharmacology, Muhammad Institute of Medical and Allied Sciences, Multan, Pakistan, after approval from the institutional ethical committee (No. 25/DPT/MIMAS/Oct/21) following the NIH guidelines for the use of animals in experimentation [36, 37].

Preliminary phytochemical evaluation

Aqueous-methanolic (30:70) leaf extract of *M. indica* was evaluated for the possible presence of vital phytochemical classes using standard protocol [32].

High-performance liquid chromatography (HPLC) analysis of aqueous-methanolic leaf extract of M. indica

The polyphenols in the aqueous-methanolic leaf extract of *M. indica* were measured with HPLC. A binary gradient solvent system was used in HPLC, paired with a C-18 column with dimensions (250 4.6 mm), capable of separating one polyphenol (mangiferin) and two flavonoids (quercetin and isoquercetin) in 36 minutes at a flow rate of 0.0008 µL/min, and a film thickness of 5 µm, with an oven set at 30°C. The replicability for separation of components was good with run-to-run. Mangiferin, quercetin, and isoquercetin were prepared as reference (purity > 99%), obtained from Aldrich (St. Louis, USA), and the dilutions were prepared with methanol to achieve 50 µg/mL. Samples were distinguished by comparing the sample retention times to standards [32]. The separation factor and resolution were used to evaluate the efficiency of separated components using HPLC.

Antioxidant activity

Antioxidant activity was analyzed using 2,2-diphenylpicrylhydrazyl (DPPH) and Nitric oxide (NO) assay.

DPPH assay

As mentioned earlier, the DPPH test has been carried out [5]. The diluted sample with methanol was mixed with an aqueous-methanolic leaf extract (30:70) from *M. indica* to make a final volume of 5 mL with different concentrations (4 mL). Then, for 40 minutes, this mixture was stored in the dark. The stated solution's 517 nm absorbance was measured using a spectrophotometer. Each study was done three times, and the percentage of inhibition in vitamin C equivalency was measured [38-41]. Equation (2) was used to compute the percentage of DPPH scavenging potential: 1% = A (blank) - B (sample)/A (blank) × 100 (2)

Nitric oxide radical scavenging assay

The extract was constructed using a 10 mg/mL aqueous-methanolic leaf extract of *M. indica*. The extract was then repetitively diluted with distilled water to yield concentrations ranging from 100 to 1,000 µg/mL, and the same procedure was applied to standard (Gallic acid). For experiments, solutions were kept at 4 degrees Celsius. For the reaction, a freshly prepared Griess reagent was used. 0.5 mL of 10 mM sodium nitroprusside in phosphate-buffered saline was combined with 1 mL of each extract concentration (100-1000 µg/mL) and incubated at 25 degrees Celsius for three hours. An equal volume of freshly produced Griess reagent was added to the extract. The control samples were created in the same manner as the test samples, minus the extracts, and with

an equal volume of buffer. The color tubes contained the specified amounts of extracts but lacked sodium nitroprusside. A volume of 150 uL of the reaction mixture was transferred to a 96-well plate. The absorbance at 546 nm was measured using a UV-Vis microplate reader (Alibaba, Hangzhou, China), as described in our previous correspondence [38-41]. The extract and standard inhibition percentage were calculated and recorded using the following formula (Equation 3), and the percentage of nitrite radical scavenging activity of extracts and gallic acid was calculated.

% nitrite radical scavenging activity: A (blank) - B (sample)/A (blank) × 100 (3)

Antipyretic activity

The antipyretic activity was estimated by using TAB vaccine-induced pyrexia and prostaglandin E (PGE)-induced pyrexia model in rabbits.

TAB-vaccine induced pyrexia

In this procedure, the rabbits were segregated into four groups, with five animals in each group. The control group was administered 2 ml/kg of normal saline. A clinical thermometer was used to measure the mean rectal temperature of a group of rabbits at an hourly interval for four hours. 0.5 ml/rabbit of typhoid vaccine (0.5 mL/rabbit) was injected intravenously into the marginal ear vein of rabbits. Aqueous-methanolic leaf extract of *M. indica* was given orally at doses of 100 and 200 mg/kg/h after TAB vaccination administration in the presence of severe fever [2, 42]. The rectal temperature was then recorded every hour for the next four hours. Paracetamol (100 mg/kg/po) was utilized for comparison.

PGE-induced pyrexia

The rabbits were sorted into four groups of five each. For 24 hours, they were kept at a constant temperature of 24°C-25°C. The animals were fasted overnight. Pyrexia was induced by S.C. injection of 100 g/kg PGE1 (misoprostol). After recording the baseline rectal temperature with a temperature sensor, after 1 hour of PGE1 injection, the aqueous-methanolic leaf extract of *M. indica* was administered orally through oral gavages. Normal saline was given to Group-1 at a rate of 2 ml/kg, aspirin 150 mg/kg was given to Group-2, while Group-3 and Group-4 were given aqueous-methanolic leaf extract of *M. indica* at a dose of 100 and 200 mg/kg.

Anti-inflammatory activity

In this study, we opted for two well-recognized inflammatory models. Models were used for acute inflammation (arachidonic acid-induced inflammation) and chronic inflammation (formalin-induced paw edema).

Arachidonic acid-induced inflammatory model

In brief, this model is primarily used to assess the anti-inflammatory potential of plant extracts and pharmaceutical drugs by causing cutaneous inflammation. Arachidonic acid provides useful information about anti-inflammatory drugs used to treat topical inflammation. Applying arachidonic acid to a specific area of the skin promotes inflammation via eicosanoids such as leukotriene C_4 (LTC_4), prostaglandin-E_2 (PGE_2), and thromboxane A_2. Because eicosanoids trigger histamine release via mast cell destruction. This inflammation is characterized by edema, severe erythema, and neutrophil accumulation. Arachidonic acid 0.01 mL topical was given to the left ear of rats while the right ear was left as control [43]. Rats were divided into 5 group: group-1 animals were used as controls, group-2 animals were given diclofenac gel 1% w/w, group-3 animals were treated with diclofenac gel 2.3% w/w, group-4 animals were treated with aqueous methanolic leaf extract of M. indica 10%, and group-5 were animals were treated with M. indica 20%. The percent decrease in edema was calculated with the help of this formula: % Inhibition = control - treated/control × 100 (4)

Formalin-induced edema model

In brief, formalin (5%) 0.1 mL sub planter injections were given to the left hind paw of rats while the right hind paw was left as control. Rats were divided into 5 groups: group-1 animal were sued as controls, group-2 animals were given diclofenac gel 1% w/w, group-3 animals were treated with diclofenac gel 2.3% w/w, group-4 animals were treated with aqueous methanolic leaf extract of *M. indica* 10%,

Table 1. Phytoconstituents present in aqueous-methanolic leaf extract of the *Mangifera indica*

Serial Number	Test	Observations	Result
1	Alkaloid	Ppt	Positive
2	Saponins	1 cm froth	Positive
3	Tannins	Light purple	Positive
4	Anthraquinones	Pink	Positive
5	Coumarins	Yellow fluorescence	Positive
6	Phenols	Light purple	Positive
7	Flavanoids	Light yellow colour	Positive

• Ppt: Precipitates; cm: Centimeter.

and group-5 were animals treated with *M. indica* 20% [44]. The percent decrease in edema was calculated with the help of this formula: % Inhibition = control - treated/control × 100 (5)

Statistical analysis

The results were communicated using the GraphPad Prism version 8 software as mean ± SEM and investigated using one-way ANOVA followed by Dunnett's multiple comparison test. The confidence interval was 95%, and $P < 0.05$ considered as significant.

Results

Preliminary phytochemical evaluation

Preliminary phytochemical evaluation of *M. indica* showed the presence of vital phytochemcial classes such as, alkaloids, terpenoids, phytosterols, flavonoids and flavonones as shown in **Table 1**.

HPLC analysis

HPLC of aqueous-methanolic leaf extract of *M. indica* was displayed in **Figure 1**. Based on retention time to standards, the main phytochemicals found were mangiferin (A), quercetin (B), and isoquercetin (C).

Antioxidant assay

DPPH assay: DPPH activity of ascorbic acid and *M. indica* is shown in **Figure 2**.

NO scavenging assay: NO scavenging assay of Gallic acid and *M. indica* is shown in **Figure 3**.

Antipyretic activity

PGE-induced pyrexia model: At a dose of 100 mg/kg b.w., the aqueous-methanolic leaf extract of *M. indica* demonstrated considerable ($P \leq 0.05$) antipyretic efficacy, with a significant drop in body temperature lasting up to 4 hours after delivery. At a 200 mg/kg dose, it demonstrated highly significant ($P \leq 0.000$) efficacy compared with the standard antipyretic drug aspirin, as shown in **Figure 4**.

TAB vaccine-induced pyrexia model: Once the extract was given to rabbits with an established TAB vaccine-induced hyperthermia, the fever was considerably lowered. The rabbits' body temperatures returned to normal when they were given 100 and 200 mg/kg of the extract orally. **Figure 5** shows that the response at higher doses was identical to that of the standard antipyretic drug paracetamol.

Anti-inflammatory activity

Acute (arachidonic acid-induced ear edema) and chronic (formalin-induced paw edema) inflammatory models were tested, and the results were explained respectively.

Arachidonic acid induced edema model: Anti-inflammatory potential of aqueous-methanolic leaf extract of *M. indica* against arachidonic acid-induced inflammation in rats is shown in **Figure 6**.

Formalin-induced paw edema model: Anti-inflammatory potential of aqueous-methanolic leaf extract of *M. indica* against formalin induced inflammation in rats is shown in **Figure 7**.

Proposed mechanism of action

Mangiferin has anti-inflammatory and analgesic activity by decreasing the synthesis of PGE-2 and COX-2 protein induced by LPS but does not modify the transcription of COX-2 [45]. It can also decrease the plasma levels of IL-1β, TNF-α, IL-6, and MCP-1 (monocyte chemoattractant protein-1) [46, 47].

Quercetin has anti-inflammatory activity by inhibiting PGE-2, cytokine, and iNOS (inducible nitric oxide synthase) by inhibiting NF-kappa B and TNF-α [48, 49]. Isoquercetin proved to be,

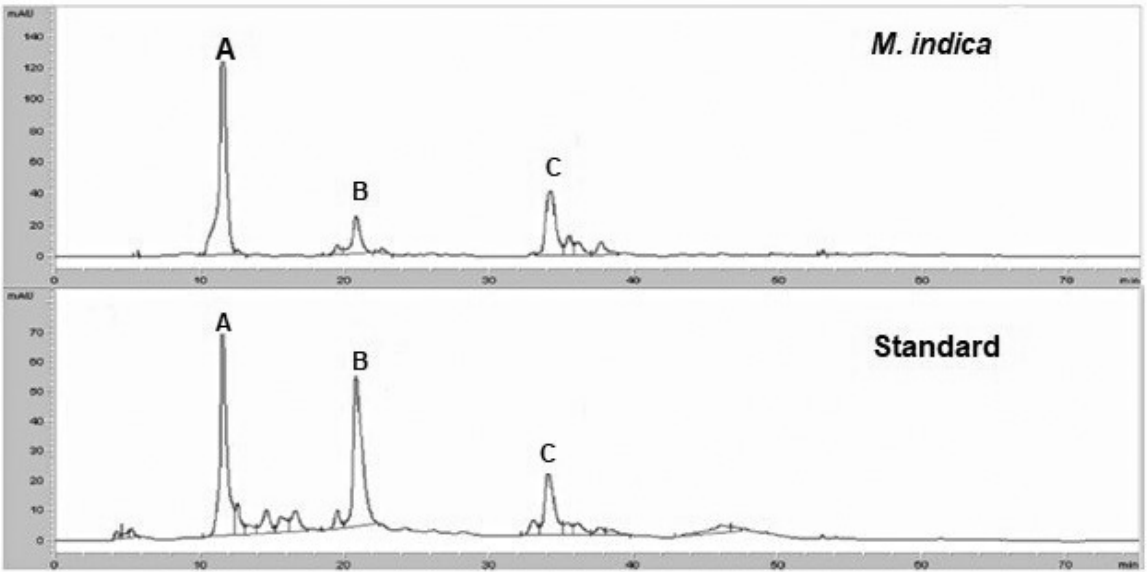

Figure 1. HPLC of aqueous-methanolic leaf extract of *M. indica* indicating the presence of mangiferin (A), quercetin (B) and isoquercetin (C) in comparison to retention time.

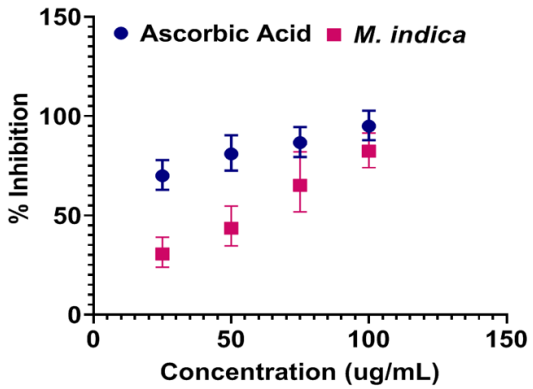

Figure 2. Antioxidant potential of aqueous-methanolic leaf extract of *M. indica* using DPPH assay concerning ascorbic acid. DPPH: 2,2-diphenylpicrylhydrazyl.

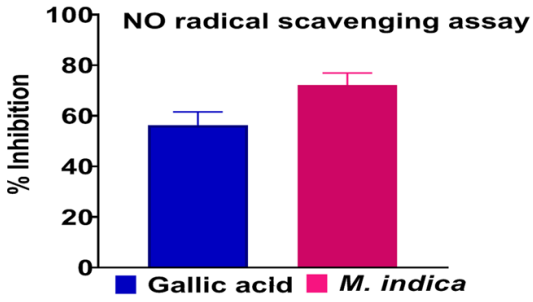

Figure 3. Antioxidant potential of aqueous-methanolic leaf extract of *M. indica* using NO scavenging assay concerning ascorbic acid. NO: Nitric oxide.

to some extent, better than quercetin for anti-inflammatory activity by inhibiting COX-2, mRNA, and inflammatory cell exudation [50] (**Figure 8**).

Discussion

Terpenoids and flavonoids were found to be the most abundant chemical components in the aqueous-methanolic leaf extract of *M. indica*, according to the early results of a phytochemical investigation (**Table 1**). Since prostaglandins are responsible for edema, flavonoids can suppress them [51]. In most cases, the antipyretic effect of nonsteroidal anti-inflammatory medications is achieved by inhibiting the synthesis of prostaglandins inside the hypothalamus [52]. The drug being tested may be able to reduce fever because it has flavonoid molecules in it. Because certain flavonoids are potent cyclooxygenase or lipoxygenase inhibitors [53], it is possible to conclude that the aqueous-methanolic leaf extract of *M. indica* lowered the body temperature by blocking the production of prostaglandins in the same way that aspirin does. *M. indica*'s antipyretic activity may be due to its phytoconstituents, flavonoids, as it has been reported to reduce the availability of prostaglandins.

Body thermal regulation demands a careful balance between heat generation and heat loss in

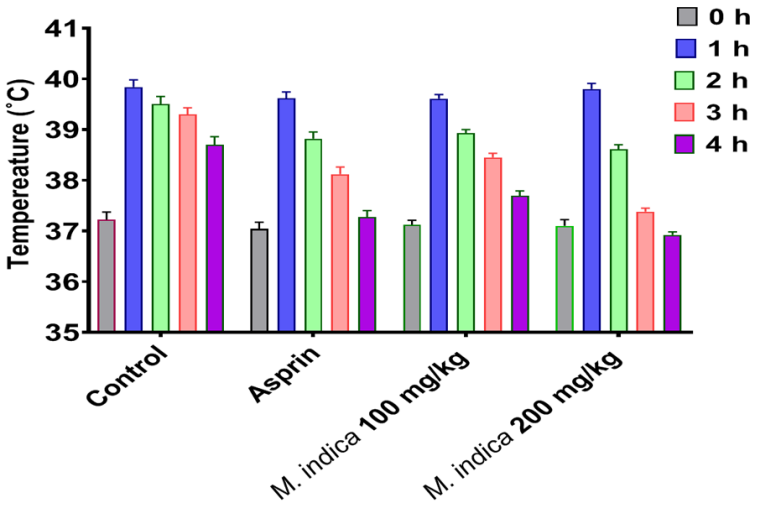

Figure 4. Antipyretic potential of aqueous-methanolic leaf extract of *M. indica* against PGE-induced pyrexia in rabbits. PGE: Prostaglandin E.

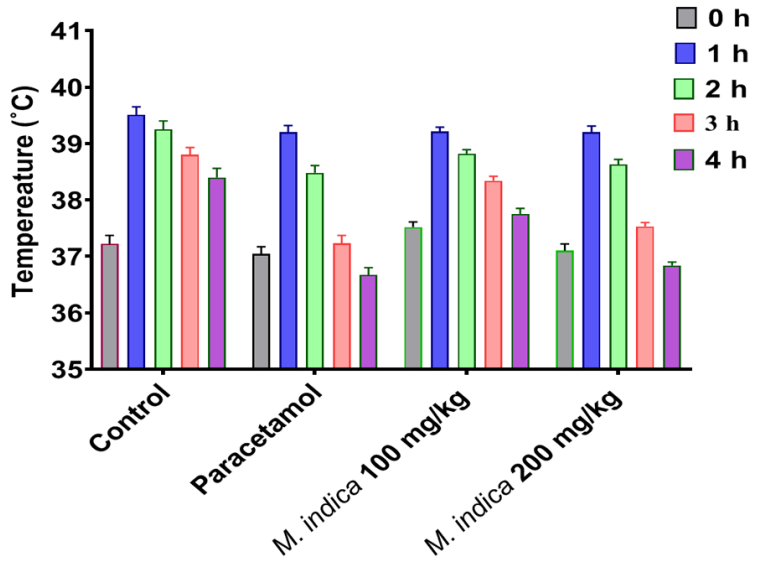

Figure 5. Antipyretic potential of aqueous-methanolic leaf extract of *M. indica* against typhoid-paratyphoid A and B (TAB) vaccine-induced pyrexia in rabbits.

the hypothalamus, which centrally maintains the set point at which body temperature is maintained [54]. The elements stated above raise this set point in fever. Antipyretic medications are known to work either centrally on the brain's temperature control center or peripherally via vasodilation and heat dissipation [2]. They reset the hypothalamic thermostat and lower fever quickly by increasing heat dissipation (sweating, cutaneous vasodilation) [52]. They also work by preventing the production of prostaglandin E2 [55]. The results indicate that the aqueous-methanolic leaf extract of M. indica has an antipyretic property comparable to that of aspirin (standard drug) in rabbits with PGE1-induced elevations in body temperature. As a result, inhibition of prostaglandin synthesis could be a plausible mechanism of *M. indica*'s antipyretic activity as aspirin [7], and there are multiple mediators or processes underlying fever pathogenesis. Any of these mediators may be inhibited from producing antipyresis. We conclude from the above study that *M. indica* has antipyretic potential against both pyrexia models in rabbits.

The arachidonic acid model is primarily used to assess the anti-inflammatory potential of plant extracts and pharmaceutical drugs by causing cutaneous inflammation. Arachidonic acid (acute inflammatory model) provides valuable information about anti-inflammatory drugs used in the treatment of topical inflammation. Applying arachidonic acid to a specific area of the skin promotes inflammation via eicosanoids such as leukotriene C_4 (LTC_4), prostaglandin-E_2 (PGE_2), and thromboxane A_2 [43]. Eicosanoids trigger histamine release via mast cell destruction. This inflammation is characterized by edema, severe erythema, and neutrophil accumulation. Formalin-induced paw edema model is used to assess the chronic anti-inflammatory efficacy of various medications. This model is identical to human arthritis [44]. Formalin causes inflammation that happens in two stages. The first stage is neurogenic and is controlled by substance P and bradykinin. Prostaglandins, serotonin, histamine, and bradykinin are all involved in the later phase [56]. Drugs such as opioids

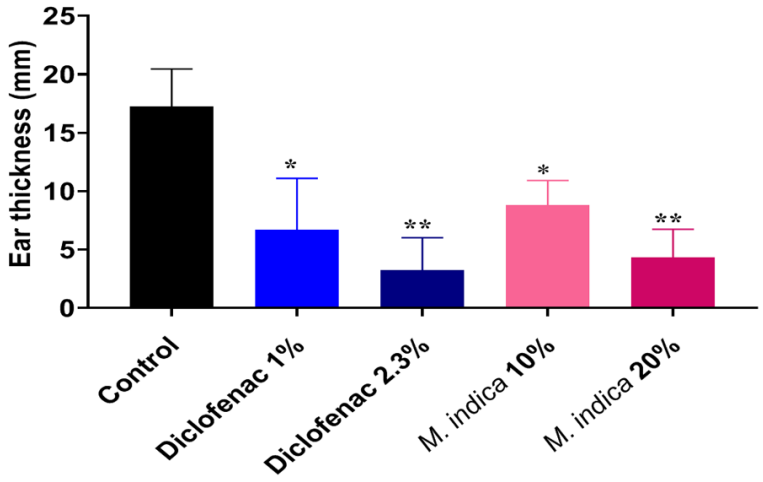

Figure 6. Anti-inflammatory potential of aqueous-methanolic leaf extract of *M. indica* against arachidonic acid-induced inflammation in rats. * = Significant variation as compared to week zero ($P < 0.05$). ** = Significant variation as compared to week zero ($P < 0.01$). NS = Non-significant variation as compared to week zero ($P > 0.05$).

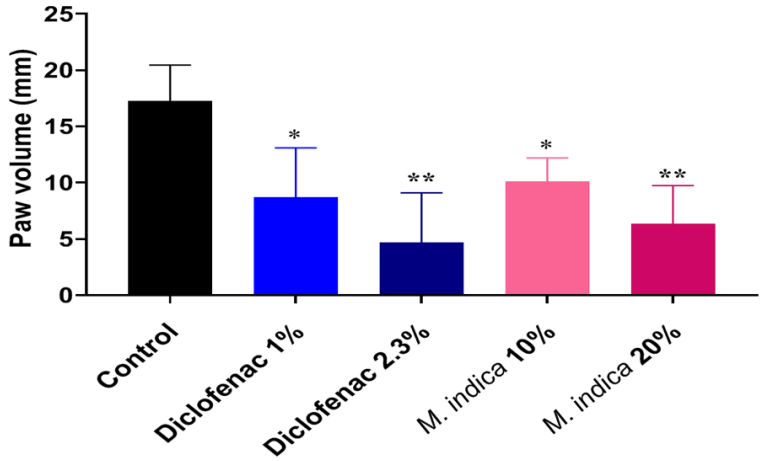

Figure 7. Anti-inflammatory potential of aqueous-methanolic leaf extract of *M. indica* against formalin induced inflammation in rats. * = Significant variation as compared to week zero ($P < 0.05$). ** = Significant variation as compared to week zero ($P < 0.01$). NS = Non-significant variation as compared to week zero ($P > 0.05$).

decrease both phases, but NSAIDs and corticosteroids inhibit the second phase. HPLC analysis of an aqueous-methanolic leaf extract of M. Indica confirmed the presence of three vital anti-inflammatory phytoconstituents: mangiferin, quercetin, and isoquercetin (**Figure 1**). The antipyretic and anti-inflammatory roles of these phytoconstituents are now well established [57], and the reported mechanism of action behind these phytoconstituents' antipyretic and anti-inflammatory properties is explained in **Figure 6**. The anti-inflammatory and antipyretic activity of these phytoconstituents corresponds to their antioxidant and antihistaminic properties.

Conclusions

Results demonstrated that the aqueous-methanolic leaf extract of *M. indica* has, antioxidant, anti-inflammatory, and antipyretic activities that may be due to different phytochemical constituents present in *M. indica* like mangiferin, quercetin, and isoquercetin. This report is the first of its kind and shows the advantage of acting on different pharmacological and biochemical pathways controlling inflammation and pyrexia.

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Disclosure of conflict of interest

None.

Abbreviations

DPPH, 2,2-diphenylpicrylhydrazyl; NO, nitric oxide; TAB, typhoid-paratyphoid A and B; PGE, prostaglandin E; HPLC, high-performance liquid chromatography; NSAIDs, nonsteroidal anti-inflammatory agents; CHD, chronic heart disease; LTC_4, leukotriene C_4; PGE_2, prostaglandin-E_2.

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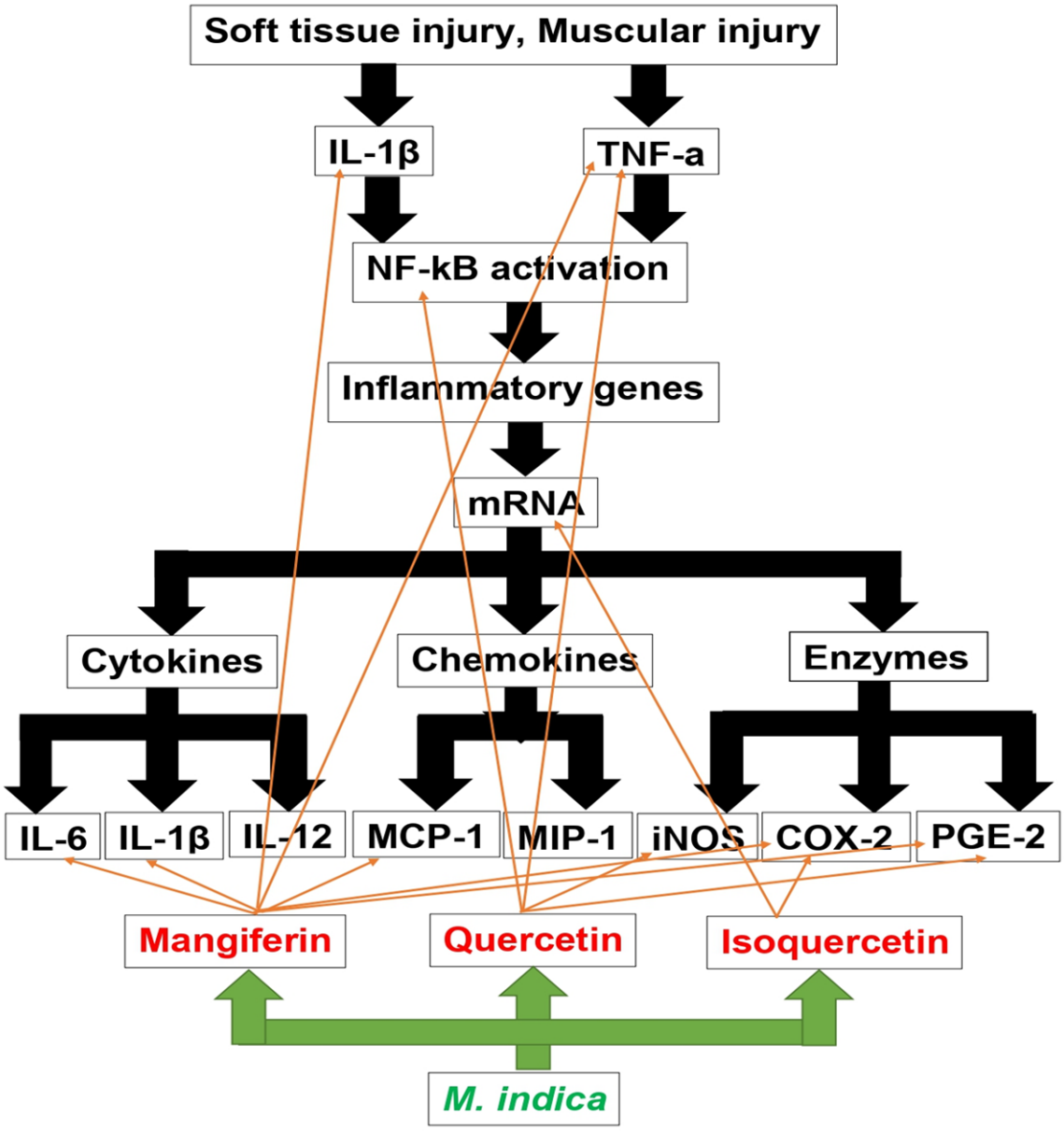

Figure 8. The proposed mechanism of action of active constituents of *M. indica* (mangiferin, quercetin and isoquercetin) on various parameters.

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